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Cast Films from Soy Protein Isolates and Fractions[1]

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Cereal Chem. 74(2):115–118

ABSTRACT

Glycerol-plasticized soy protein films were cast from alkaline aqueous film-forming solutions of laboratory-prepared 7S, 11S, and soy isolate (LSI) fractions and from commercial soy isolate (CSI). Tensile strength (TS), elongation at break (E), water vapor permeability (WVP), total soluble matter (TSM), protein solubility (PS), and Hunter *L*, *a*, and *b* color values of these films were determined. The 11S films had greater TS than 7S films ($P < 0.05$), while LSI films had greater TS than CSI films ($P < 0.05$). No significant differences were detected among mean E values and among mean WVP values of all films ($P > 0.05$). The 7S films had higher TSM and PS values than 11S films ($P < 0.05$). CSI films were significantly darker (lower *L* value) and more yellow (greater positive *b* value) than LSI films ($P < 0.05$).

Edible films and coatings can prolong the shelf life of foods due to their selective barrier properties against movement of moisture, gases, and vapor. Interest in edible packaging is likely to grow in the near future as it has potential for reducing the use of nonedible or synthetic packaging (Gennadios et al 1993a). Kester and Fennema (1986) discussed edible films derived from several polysaccharides, lipids, and proteins. Formation and properties of films from proteins such as casein, whey protein, corn zein, wheat gluten, and soy protein were reviewed by Gennadios et al (1994a).

The film-forming ability of soy proteins has traditionally been utilized in the Far East for production of soy protein-lipid films called *yuba films* (Wang 1981). The process of yuba film formation consists of boiling soy milk in shallow pans; collecting the films formed (due to surface dehydration) by means of rods; and hanging the films to air-dry (Gennadios and Weller 1991, Brandenburg et al 1993). Jaynes and Chou (1975) developed a method to prepare soy films by spreading soy protein isolate solutions on Teflon-coated baking pans followed by baking the pans at 100ºC for 1 hr. Deposition and drying of soy protein solutions is a more promising method for commercial-scale film production than the traditional yuba film process, since it allows for more consistency and control during film formation (Gennadios and Weller 1991). Gennadios et al (1993a) described a method to develop homogeneous free-standing edible films from commercial isolated soy protein using glycerol as a plasticizer.

Soybean protein consists of two major globulin fractions, the 7S (β-conglycinin) and the 11S (glycinin), which make up ≈37 and 31%, respectively, of the total extractable proteins (Gennadios et al 1994a). Thanh and Shibasaki (1976) found that the 7S and 11S fractions constituted 48 and 35%, respectively, of total protein. The 7S protein is extensively glycosylated and can assume as many as seven different forms due to combinations of its three peptide subunits a, a′, and b (Iwabuchi and Yamauchi 1987). On the other hand, the 11S protein does not contain an appreciable amount of carbohydrate, and its subunits differ in charge and molecular weight (Iwabuchi and Yamauchi 1987). These structural differences contribute to variations in the functional properties of 7S and 11S fractions (Romijn et al 1991). For instance, the 11S fraction has a more significant impact on gelation characteristics of soy protein than does the 7S fraction (Nakamura et al 1985). Film formation on the surface of heated aqueous solutions of 7S and 11S soy proteins, similar to the formation of yuba films on the surface of heated soy milk, has been reported (Shirai et al 1974, Okamoto 1978). However, no information can be found in the literature related to cast films from 7S and 11S soy protein fractions.

The purpose of this study was to compare the tensile strength, elongation at break, water vapor permeability, solubility in water, and color properties of films formed from commercial soy protein isolate to those of films formed from laboratory-prepared crude 7S, crude 11S, and soy protein isolate.

MATERIALS AND METHODS

Reagents

Defatted, low-heat treated soy flakes were obtained from Archer Daniels Midland Co. (Lincoln, NE). Glycerol was purchased from Fisher Scientific Co. (Pittsburgh, PA). A food grade soy protein isolate (SUPRO 620) was obtained from Protein Technologies International (St. Louis, MO). Sodium hydroxide, hydrochloric acid, 2-mercaptoethanol, Tris-HCl buffer, sodium azide, and bicinchoninic acid (BCA) protein assay kit were purchased from Sigma Chemical (St. Louis, MO). Sodium dodecyl sulfate (SDS), acrylamide-bisacrylamide, ammonium persulphate, tetramethylethylenediamine (TEMED), bromophenol blue, and coomassie brilliant blue R-250, all of electrophoresis grade, were purchased from Fisher Scientific Co. (Pittsburgh, PA). Boric acid and sodium hydroxide required for protein determination by the Kjeldahl method were purchased from Ricca Chemical Co. (Arlington, TX), sulfuric acid was purchased from Baxter (McGraw Park, IL), and the catalyst tablets were purchased from Fisher Scientific Co. (Pittsburgh, PA).

Protein Isolation

Soy protein isolate was prepared from finely ground, low-heat treated, defatted soy flakes as described by Romijn et al (1991). The soy globulin fractions 7S and 11S were isolated from the same source (defatted soy flakes) using a simultaneous fractionation method developed by Thanh and Shibasaki (1976). This method was based on the differential solubilities of the two globulin fractions at different pH values. Pellets of crude 7S, crude 11S, and soy protein isolate were redissolved separately in 0.03*M* Tris-HCl buffer at pH 7.8 (1:40 protein pellets to buffer ratio) and dialyzed overnight using Spectra/Por molecular porous dialysis tubing with a molecular weight cutoff of 6,000–8,000 Da (Spectrum Medical Industries, Inc., Los Angeles, CA). The dia-

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Publication no. C-1997-0201-05R.

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lyzed protein solutions were frozen and freeze-dried (stoppering tray drier, model 77545, Labconco, Kansas City, MO). Temperature programming for 2,000 mL of frozen solution was –40 to 0ºC with a ramp of 0.05ºC/min and hold time of 10 hr for each stage. The resultant white, fluffy, and shiny protein powder was stored at –20ºC until needed.

Gel Electrophoresis

Purities of the laboratory-prepared 7S, 11S, and soy protein isolate were assessed using a SDS-PAGE method described by Hames and Rickwood (1981). Slab gel, discontinuous SDS-PAGE was performed using a Mini-PROTEAN II electrophoresis cell (Bio-Rad Laboratories, Hercules, CA). A 10% resolving gel solution was prepared by mixing 33.33% (v/v) acrylamide-bisacrylamide (30:0.8); 12.5% (v/v) 1.5*M* Tris-HCl buffer (pH 8.8); 1% (v/v) of 10% SDS solution; and 4.66% (v/v) of 1.5% ammonium persulphate solution in water. The stacking gel solution was prepared by mixing 12.5% (v/v) acrylamide-bisacrylamide (30:0.8); 25% (v/v) 0.5*M* Tris-HCl buffer (pH 6.8); 1% (v/v) of 10% SDS solution; and 5% (v/v) of 1.5% ammonium persulphate. Both gel solutions were polymerized using 0.05% (v/v) TEMED. Protein samples were prepared by heating ≈3 mg of protein in 1 mL of a solution of 2% SDS, 5% 2-mercaptoethanol, 0.002% bromophenol blue, and 10% glycerol in 0.625*M* Tris-HCl buffer (pH 6.8). About 10 μg of each protein solution was loaded onto the gel. Electrophoresis was performed at constant 200V until the dye front reached the bottom. Gels were stained in a solution of 0.1% (w/v) coomassie brilliant blue R-250 in 50% methanol and 40% distilled water and 10% glacial acetic acid (v/v). Gels were destained using 50% methanol and 40% distilled water and 10% glacial acetic acid (v/v).

Protein Content Determination

Protein content of oven-dried (130ºC for 4 hr) laboratory-prepared crude 7S, 11S, and soy protein isolate fractions and commercial soy protein isolate powder were determined in triplicate according to AOAC method 981.10 (AOAC 1990) using a 6.25 nitrogen conversion factor.

Film Preparation

Film-forming solutions were prepared by stirring commercial soy protein isolate (CSI), laboratory-prepared soy protein isolate (LSI), crude 7S, or crude 11S protein (5%, w/v) and glycerol (1.5%, w/v) in distilled water for 15 min. The pH values of the solutions were adjusted to 10.0 ± 0.1 with 2*N* sodium hydroxide. Subsequently, the solutions were heated in a water bath (Magni Whirl, Blu M Electric Company, Blue Island, IL) at 85ºC for 30 min, filtered through cheese cloth (grade 40, Fisher Scientific, Pittsburgh, PA) to remove foam and undissolved impurities and poured (25 mL) in rectangular areas (10 × 18 cm) created with thin plastic strips glued to leveled (to the nearest of 0.01 mm) Teflon-coated glass plates. Cast solutions were allowed to dry at room temperature (25ºC) for ≈15 hr and were then peeled off the plates.

Thickness Measurement

Thickness was measured to the nearest 2.54 μm using a handheld micrometer (B.C. Ames Co., Waltham, MA). Thickness of individual samples for water vapor permeability measurement were determined by averaging five measurements taken one at the center and four on the perimeter of a 7- × 7-cm film specimen. For film strips used for tensile testing (10 × 2.5 cm), thickness was determined as a mean of five measurements taken along the strips.

Tensile Testing

All films were conditioned for 48 hr in an environmental chamber at 25ºC and 50% rh before testing. Tensile testing was performed with a Universal Testing Instrument (model 5566, Instron Engineering Corp., Canton, MA) on 10 × 2.5 cm rectangular film strips. Initial grip separation was set at 50 mm, while cross-head speed was set at 500 mm/min. Tensile strength (TS) was calculated by dividing maximum (peak) load developed during the test by initial film specimen cross-sectional area. Percentage elongation at break (E) was calculated by dividing film extension at the moment of rupture with 50 mm (initial gauge length) and multiplying by 100.

Total Soluble Matter

Total soluble matter (TSM) of films was determined using a method described by Gontard et al (1992). Initial dry matter of film pieces (20 × 20 mm) was measured by drying in an air-circulating oven at 100ºC for 24 hr. These film pieces were placed in beakers containing 50 mL of distilled water and traces of sodium azide (0.02%, w/v) to prevent microbial growth and stored at room temperature for 24 hr with occasional gentle stirring. Undissolved dry film matter was determined by taking the film pieces out of the beakers and drying them in an air-circulating oven (100ºC for 24 hr). The weight of solubilized dry matter was calculated by subtracting the weight of unsolubilized dry matter from the initial weight of dry matter and reported on an initial dry weight basis.

Protein Solubility

Small pieces of films (≈1 g) were placed in a desiccator over calcium sulfate (0% rh) for 72 hr. Dried film samples (10–20 mg) were accurately weighed and placed into test tubes, to which 5 mL of distilled water were added. Test tubes were covered with wrapping film (Parafilm M, American National Can, Greenwich, CT) and incubated at room temperature for 24 hr. Tubes were gently shaken and 1 mL of the solution was put into microfuge tubes and centrifuged at 2,000 rpm for 10 min. A 100-μL sample of the supernatant was analyzed for protein content with the bicinchoninic acid (BCA) protein assay kit (Sigma procedure no. TPRO-562, Sigma Chemical Co., St. Louis, MO) using bovine serum albumin as the standard for protein quantitation (Smith et al 1985). Protein solubility (PS) results were reported as the percentage of total protein detected in solution. The initial amount of protein in the dry films was calculated from the initial dry film weight after accounting for both the protein to glycerol ratio in the film and the protein content of the LSI, CSI, 7S, or 11S powder.

Water Vapor Permeability

Water vapor permeability (WVP) of films was determined gravimetrically using methodology similar to that described by Gennadios et al (1994b). Cups with an open mouth area of 16.6 cm^2 were filled with 15 mL of distilled water, and film specimens (7 × 7 cm) were tightly sealed on the cups by means of a lid, four screws, and a rubber O-ring. The cup assemblies were weighed and placed in an environmental chamber at 50% rh and 25ºC. Air circulation inside the chamber was provided by a fan (198 m/min air speed). Moisture loss was monitored by weighing the cups at 1-hr intervals during an 8-hr period. Steady state was reached within 2 hr. Linear regression-derived slopes of the steady state (linear) portion of weight loss versus time curves were used to estimate water vapor transmission rate (WVTR) in $g/m^2 \times hr$. WVP ($g \times mm/m^2 \times hr \times kPa$) was calculated as: $WVP = WVTR(L/\Delta P)$, where L was film thickness (mm) and ΔP was partial water vapor pressure difference between the two sides of the film (kPa).

Color Measurement

Color values of soy protein films were measured with a chroma meter (CR-300, Minolta Camera Co., Osaka, Japan). Film specimens were placed on the surface of a white standard plate (calibration plate CR-A43) and Hunter *L*, *a*, and *b* color values were measured. The three color coordinates ranges were: *L* (0 black to 100 white), *a* (–greenness to + redness), and *b* (–blueness

to + yellowness) (Francis and Clydesdale 1975). Total color difference (ΔE) was calculated as:

$$\Delta E = [(L_{\text{film}} - L_{\text{standard}})^2 + (a_{\text{film}} - a_{\text{standard}})^2 + (b_{\text{film}} - b_{\text{standard}})^2]^{0.5}$$

Standard values refer to the white calibration plate (L = 96.86, a = –0.02, and b = 1.99).

Statistical Analysis

Measurements of each property were replicated three times for each type of film, with individually prepared and cast films as the replicated experimental units. Each property replicate was the mean of two tested sampling units taken from the same film. Statistics on a completely randomized design were determined using the General Linear Models procedure in the SAS program (SAS 1988). Mean property values were separated ($P < 0.05$) with Duncan's multiple range test (Steel and Torrie 1980).

RESULTS AND DISCUSSION

Gel Electrophoresis

SDS-PAGE gels prepared in this study are shown in Figure 1. Both laboratory-prepared 7S and 11S fractions had relatively high purity. The level of cross-contamination between the two fractions was typical for the isolation technique used (Thanh and Shibasaki 1976, Winters et al 1990). Similar electrophoretic patterns of protein subunits were obtained for CSI and LSI.

Tensile Strength and Elongation

Mean TS and E values for 7S, 11S, CSI, and LSI films are presented in Table I. Films made from 11S soy globulin fraction had significantly ($P < 0.05$) greater TS, by ≈46%, than did films from 7S. Similarly, it has been previously reported that yuba-like films (formed on the surface of heated soy protein solutions) from 11S were stronger than films from 7S (Shirai et al 1974, Okamoto, 1978). Covalent disulfide bonds are believed to play a major role in soy protein film formation (Gennadios and Weller 1991). Therefore, the higher tendency of 11S protein to form disulfide bonds than 7S protein (Saio et al 1971) could be responsible for 11S films being stronger. LSI films had greater ($P < 0.05$) TS than did CSI films (Table I). This was attributed to LSI powder having a higher protein content (94.4%, db) than CSI powder (91.5%, db), since nonprotein constituents are likely to disrupt the homogeneity and continuity of the protein film network. In agreement with this, Wall and Beckwith (1969) showed that commercial wheat gluten did not yield as strong a film as laboratory-prepared wheat gluten. Also, Gennadios and Weller (1992) reported that films prepared from a 75% (db) protein content commercial wheat gluten had lower TS than that of films prepared from an 82% (db) protein content commercial wheat gluten.

In contrast to TS, no significant ($P > 0.05$) differences were detected among mean E values of 7S, 11S, CSI, and LSI films (Table I). It should be noted that film thickness was not accounted for in calculation of E values as was the case with calculation of TS values. Therefore, the substantial variability in measured E values and failure to detect differences among mean values may have been due to the small thickness variations of tensile testing film specimens.

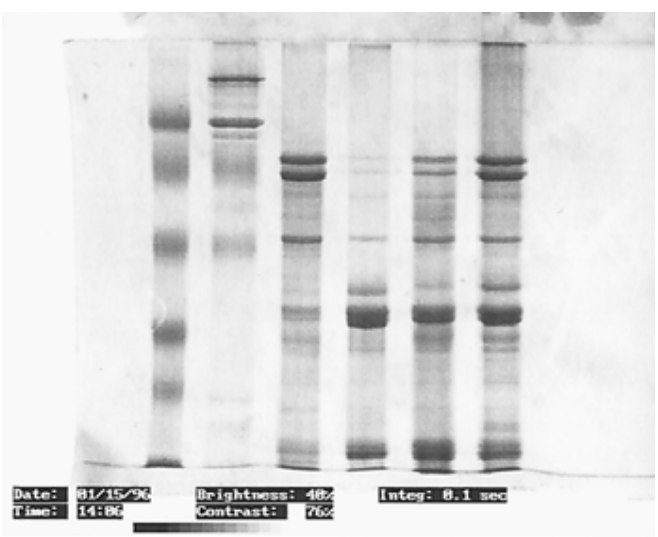

Fig. 1. Sodium dodecyl sulfate polyacrylamide gel electrophoresis patterns for laboratory-prepared 7S soy protein fraction (lane 3 from left); laboratory-prepared 11S soy protein fraction (lane 4); laboratory-prepared soy protein isolate (lane 5); and commercial soy protein isolate (lane 6).

Solubility in Water

Stuchell and Krochta (1994) reported that pieces of glycerol-plasticized films from commercial soy protein isolate maintained their integrity after incubation in water for 24 hr with occasional gentle agitation. They suggested that only monomers, small peptides, and nonprotein materials solubilized in water. Film pieces immersed in water in the present study were not broken apart and the film network remained intact. Most likely, the hydrophilic glycerol constituted a large part of the film TSM.

TABLE I
Tensile Strength (TS), Percent Elongation at Break (E), Protein Solubility (PS), and Total Soluble Matter (TSM) of Soy Protein Films[a]

Film	TS (MPa)	E (%)	PS (%)	TSM (%)
7S	7.36 ± 0.42a	37.06 ± 18.5a	8.59 ± 0.20c	39.41 ± 1.56d
11S	10.77 ± 0.63b	37.93 ± 18.5a	3.99 ± 0.05a	26.81 ± 0.16a
CSI[b]	8.53 ± 0.48a	31.93 ± 2.4a	6.51 ± 0.26b	35.11 ± 0.99c
LSI[c]	10.68 ± 0.95b	41.66 ± 22.0a	12.19 ± 0.30d	29.91 ± 1.95b

[a] Means of three replicates ± a standard deviation. Any two means in the same column followed by the same letter are not significantly ($P > 0.05$) different according to Duncan's multiple range test.
[b] Commercial soy protein isolate.
[c] Laboratory-prepared soy protein isolate.

TABLE II
Water Vapor Permeability (WVP)[a] of Soy Protein Films at 25°C

Film	Thickness (μm)	WVP (g × mm/m^2 × hr × kPa)	rh[b] (%)
7S	65 ± 4.5	4.39 ± 0.59	73.14 ± 0.76
11S	57 ± 7.0	4.27 ± 0.54	71.82 ± 0.16
CSI[c]	54 ± 6.0	3.76 ± 0.16	73.26 ± 1.20
LSI[d]	62 ± 6.1	3.88 ± 0.30	74.00 ± 0.53

[a] Means of three replicates ± a standard deviation. No significant ($P > 0.05$) differences were detected among WVP means.
[b] Actual rh values at the underside of films calculated as described by Gennadios et al (1994b) to account for resistance of stagnant air layer between film and water surface in testing cups. rh outside the cups was 50%.
[c] Commercial soy protein isolate.
[d] Laboratory-prepared soy protein isolate.

TABLE III
Hunter Color Values (*L*, *a*, and *b*) and Total Color Differences (ΔE) of Soy Protein Films[a]

Film	*L*	*a*	*b*	ΔE
7S	94.25 ± 0.35a	–2.42 ± 0.25a	11.64 ± 1.05ab	10.28 ± 1.12ab
11S	94.83 ± 0.44a	–3.16 ± 0.16b	10.66 ± 0.70b	9.55 ± 0.68b
CSI[b]	93.05 ± 0.40b	–2.46 ± 0.34a	12.62 ± 1.43a	11.56 ± 1.50a
LSI[c]	94.77 ± 0.16a	–2.30 ± 0.18a	9.93 ± 0.87b	8.53 ± 0.79b

[a] Film color was measured by placing films on top of a standard white plate (L = 96.86, a = –0.02, and b = 1.99). Presented values are means of three replicates ± a standard deviation. Any two means in the same column followed by the same letter are not significantly ($P > 0.05$) different according to Duncan's multiple range test.
[b] Commercial soy protein isolate.
[c] Laboratory-prepared soy protein isolate.

Films from 7S had higher ($P < 0.05$) TSM and PS values than 11S films (Table I). This was attributed to the lower molecular weight of 7S proteins, the higher tendency of 11S proteins to form disulfide bonds (Saio et al 1971), which stabilize the film network, and the more lipophilic nature of 11S proteins (Watanabe et al 1975). The higher ($P < 0.05$) TSM of CSI films compared to LSI films (Table I) was likely due to the presence of a greater amount of nonprotein impurities in the CSI powder. However, CSI films had lower ($P < 0.05$) PS than did LSI films.

Water Vapor Permeability

No significant ($P > 0.05$) differences were detected among WVP values of 7S, 11S, CSI, and LSI films (Table II). In general, protein films are poor water vapor barriers due to the inherent high hydrophilicity of proteins and the substantial amount of hydrophilic plasticizers added to protein films (Gennadios et al 1994a). For the sake of comparison, low density polyethylene films have WVP values (25 μm films, 90% rh gradient, 38ºC) ranging from 0.0026 to 0.0035 $g \times mm/m^2 \times hr \times kPa$ (Briston 1988), which are lower by three orders of magnitude than WVP values of soy protein films measured in the present study.

Film Color

Color is an important property of protein films because it could affect consumer acceptance of such films in potential edible or nonedible packaging applications. Hunter *L*, *a*, and *b* color values and total color differences for 7S, 11S, CSI, and LSI films are shown in Table III. CSI films were slightly but significantly ($P < 0.05$) darker (lower mean *L* value) and more yellow (greater mean positive *b* value) than the other three types of films. This was attributed to the greater amount of impurities present in the CSI powder.

Implications and Potential Applications

This study showed that soy protein isolate of increased purity can yield stronger films than commercially available soy protein isolate. Also, use of 11S soy globulin fraction alone can give stronger films than commercial soy protein isolate. However, this improvement in film strength would come at the additional costs of further purifying soy protein isolate or separating the 11S protein fraction. Although soy protein films are poor water vapor barriers, they have been found to be very effective oxygen barriers (Brandenburg et al 1993, Gennadios et al 1993b). The good oxygen barrier ability of soy protein films could be utilized in the manufacture of multilayer packagings where soy protein films would function as the oxygen barrier-providing layer. In another potential application, soy protein coatings on precooked meat products could control lipid oxidation and limit surface moisture loss. Incorporation of antioxidants and flavoring agents in soy protein coatings could improve overall quality characteristics of food products. The high hydrophilicity and substantial solubility of soy protein films in water could possibly render such films appropriate for manufacture of hot water soluble pouches, similar to the cellulose ether-based water soluble pouches which are currently in commercial use.

ACKNOWLEDGMENT

This research was partially funded by the Nebraska Soybean Development, Utilization, and Marketing Board.

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[Received June 3, 1996. Accepted November 5, 1996.]